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NOTES ON LIFE CYCLES IN COLOMBIAN ISOLATES OF *ERNODESMIS* AND *BOODLEA* (SIPHONOCLADALES, CHLOROPHYTA)*

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ABSTRACT – The formation of biflagellate swarmers (*Ernodesmis verticillata* (Kützing) Boergesen, *Boodlea composita* (Harvey & Hooker) Brand, and quadriflagellate swarmers (*Boodlea composita*) has been studied under culture conditions. Relative nuclear DNA contents of thalli and swarmers measured after Feulgen staining show that diploid *Ernodesmis verticillata* and *Boodlea composita* thalli produce diploid biflagellate zoospores. Germlings derived from these zoospores grow up to thalli corresponding to the starting material in shape and ploidy level. – In *Boodlea composita* two types of zoospores are formed by the same thallus. Diploid biflagellate zoospores become released about 1 hour after the beginning of the photoperiod, while haploid quadriflagellate zoospores [redacted] set free about 4-5 hours later. Germlings of the haploid quadriflagellate zoospores exhibit only little growth under culture conditions favourable for the diploid life cycle phases.

RÉSUMÉ – En cultures conditionnées, on [redacted] examiné le développement de zoïdes biflagellés (*Ernodesmis verticillata, Boodlea composita*) et quadriflagellés (*Boodlea composita*). Le contenu relatif en DNA des noyaux cellulaires est mesuré cytophotométriquement après coloration de Feulgen. Nos mesures montrent que les thalles diploïdes d'*Ernodesmis verticillata* et de *Boodlea composita* produisent des zoïdes biflagellés diploïdes. Les plantules issues de ces zoïdes évoluent en de nouveaux thalles qui sont, par leurs formes et leur teneur en DNA semblables aux plantes initiales. Chez *Boodlea composita* des zoïdes diploïdes et des zoïdes haploïdes se forment sur le même thalle. Les zoïdes diploïdes biflagellés sont libérés une heure après le début de la photopériode, tandis que la libération des zoïdes haploïdes quadriflagellés s'effectue 4 à 5 heures plus tard. Les plantules issues des zoïdes quadriflagellés ne présentent qu'une croissance réduite comparativement [redacted] plantules diploïdes placées dans les mêmes conditions de culture.

KEY WORDS : Siphonocladales, *Ernodesmis verticillata, Boodlea composita,* life history, biflagellate diploid and quadriflagellate haploid zoospores, nuclear DNA content, microspectrophotometry.

* This paper contents some results of the doctoral theses of S.B. and U.K.

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Source : MNHN, Paris

INTRODUCTION

A correct interpretation of morphological life history phases is not possible without data concerning the respective nuclear ploidy levels. In algae the sizes of chromosomes often represent as severe obstacle for counting. Nevertheless, relative ploidy levels may be determined by indirect methods (microspectrophotometry). By these methods for example meiosis could be localized in the fresh water alga *Batrachospermum* Roth (Rhodophyta) (Hurdelbrink & Schwantes, 1972), and it could be shown that the filamentous thallus of *Vaucheria* De Candolle (Xanthophyceae) considered to be haploid during a long time, represents a diploid life cycle phase (Al-Kubaisy *et al.*, 1981).

For a large number of species in the order Siphonocladales informations about life histories are still insufficient of entirely lacking (e. g. Tanner, 1981). Generally, the descriptions of life cycles are based on abservations of vegetative stages and swarmers, without taking into account the ploidy levels of respective nuclei. While quadriflagellate swarmers of these algae mostly are considered to be zoopsores (e. g. Iyengar & Ramanathan, 1940; Iyengar & Ramanathan, 1941; Chihara, 1955; Enomoto & Hirose, 1970) there exist controversial assumptions concerning biflagellate swarmers. The latter are thought by many authors to be gametes, an opinion supported in several cases by the observation of syngamy (e. g. Iyengar & Ramanathan, 1940; Chihara, 1965; Enomoto & Hirose, 1970) or planozygotes with two stigmata (e. g. Iyengar & Ramanathan, 1941; Chihara, 1959; Enomoto & Okuda, 1981). Others authors (e. g. Famitzin, 1860; Schechner-Fries, 1934; Jonsson & Puiseux-Dao, 1959) suppose that biflagellate swarmers may be zoospores.

Obviously, such and similar controverse interpretations of life history phases must result in contradictory life cycle concepts. An example for *Valonia* Ginnani is given by Tanner (1981, p. 235).

In this paper we present data on nuclear DNA contents and ploidy levels of biflagellate (*Ernodesmis verticillata, Boodlea composita*) and quadriflagellate swarmers (*Boodlea composita*), all identified as zoospores. Prior studies in *Ernodesmis verticillata* showed the existence of haploid biflagellate swarmers considered to be gametes capable of parthenogenetic germination; autodiploidization took place in the germlings with the consequence of a simultaneous existence of haploid and diploid nuclei in one cell. Further, the possibility of the formation of diploid mitozoospores in this species has been mentioned, but could not be proofed (Schnetter *et al.*, 1984).

Concerning the reproduction in the genus *Boodlea*, there is only one report based on *Boodlea coacta* (Dickie) Murray et De Toni from Japan (Chihara, 1955). This author observed quadriflagellate swarmers, which do not conjugate but germinate immediately after release.

MATERIAL AND METHODS

Ernodesmis verticillata was collected in February 1985 near Acandi, Depto.

Source : MNHN, Paris

Choco, Caribbean coast of Colombia, at a depth of about 3 meters.

Boodlea composita was found in August 1985 near Santa Marta, Depto. Magdalena, Caribbean coast of Colombia, growing on rocks in the upper sublittoral.

Isolates of both species were cultivated as unialgal cultures in Giessen at a temperature of 23°-27°C and at a light intensity of about 2.4 Wm^2 (12 h photoperiod) using von Stosch's culture medium (see Schnetter *et al.*, 1984).

Relative nuclear DNA contents were measured by microspectrophotometry after Feulgen staining as described by Al-Kubaisy *et al.* (1981), taking into account cloned starting material, swarmers and germlings raised in cultures. Results of measurements [redacted] given in arbitrary units (AU).

Thalli of *Ernodesmis* were prepared and fixed as described by Schnetter *et al.* (1984). *Boodlea* thalli were fixed for 45 min in ethanol:formalin:acetic acid (89:10:1 vols) at room temperature. Then they were transferred onto protein-glycerol coated microscope slides, cut open using a razorblade and spread in one layer. For the study of swarmers, fertil *Ernodesmis* and *Boodlea* cells were cut off and transferred onto protein-glycerol coated microscope slides. The swarmers leave their zoidangia through liberation tubes. This process was hastened by light pressure using a preparation needle. All samples were allowed to dry at 100°C. After short incubation in hot ethanol (96 %) to remove the chlorophyll (and for fixation of unfixed material), the objects were Feulgen stained (hydrolysis : 105 min at 20°C).

RESULTS AND DISCUSSION

1. *Ernodesmis verticillata*

Two different ranges of nuclear DNA contents have been found in thalli of *Ernodesmis* collected in Colombia and cloned in culture. In some of these strains (Fig. 1 A) the DNA contents corresponded to the diploid plants studied previously by Schnetter *et al.* (1984). In further material (Fig. 1 B) the DNA level is similar to that observed by Schnetter *et al* (1984) in thalli raised after parthenogenetic germination of gametes. Due to autodiploidization diploid [redacted] well as haploid nuclei were present in cells of these germlings after some weeks. The simultaneous existence of haploid and diploid nuclei in cells of *Ernodesmis* thalli collected in the sea (this paper) is indicating that parthenogenesis and autodiploidization observed in culture (Schnetter *et al.*, 1984) are also occurring in the natural environment.

Plants from Colombia are well growing at [redacted] temperature of 23°C. At this and higher temperatures (up to 27°C) single or all thallus cells may become transformed into zoidangia producing biflagellata swarmers 3.6 - 14.5 µm long. Nuclear DNA contents of swarmers released by thalli with haploid and diploid somatic nuclei apparently extend from haploid to diploid levels (Fig. 1 C). A fusion of haploid swarmers (assumed to be gametes) still could not be observed.

Source : MNHN, Paris

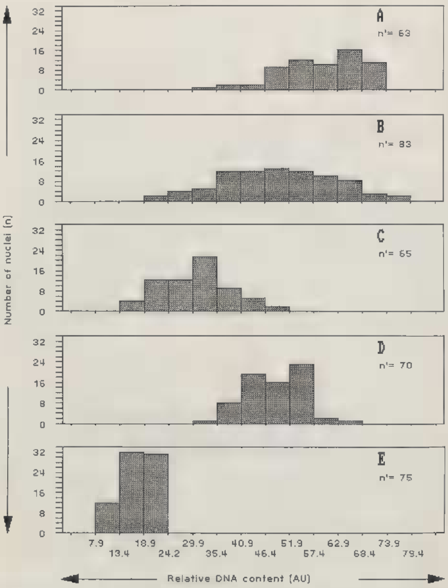

Fig. 1 — *Ernodesmis verticillata*, nuclear DNA contents expressed in arbitrary units (AU) measured after Feulgen staining; n' = total number of measured nuclei. — A. Diploid thalli; B. Thalli with haploid and diploid nuclei; C. Simultaneously produced haploid and diploid biflagellate swarmers of B; D. Diploid biflagellate swarmers (mitozoospores) of A; E. Haploid biflagellate swarmers (gametes) released by diploid thalli (the last data taken from Schnetter *et al.*, 1984).

Source : MNHN, Paris

Thalli with exclusively diploid nuclei (Fig. 1 A), matching the diploid plants studied by Schnetter *et al.* (1984, fig. 1 A) in their nuclear DNA contents produced biflagellate swarmers. The minima (G_1 stage of the mitotic cycle) of nuclear DNA contents of mother thalli cells and these swarmers (Fig. 1 D) are identical. It is concluded, that the now observed biflagellate swarmers are diploid mitozoospores. The range of nuclear DNA contents in zoospores (29.9-68.4 AU; Fig. 1 D) may indicate that their nuclei are in different stages (G_1, S, G_2) of the mitotic cycle.

Judging from the DNA contents of gamete nuclei (13.4-18.9 AU; fig. 1 E), DNA levels about 29.9-35.4 AU (see Fig. 1 C) should correspond to the G_1 stage of diploid nuclei. It is supposed that the stage of sporangial nuclei immediately before the formation of mitozoospores is neither uniform nor does have much influence in the sporogenesis.

2. *Boodlea composita*

Reticulate thallus branches characteristic for *Boodlea composita* (Egerod, 1975, fig. 19) develop at temperatures between 23° and 27°C in culture. About one month later single cells of these parts transform into zoidangia. The first signs of the incipient thallus maturation are a change in colour from light green to dark green and the development of liberation tubes in the young zoidangia (Fig. 2 A). During the next 24 hours the cytoplasma contracts into a netlike structure (Fig. 2 B) from which many swarmers arise.

An individual plant generally produces two types of zoidangia and hence two types of swarmers. In culture, about 1 hour after the beginning of the photoperiod biflagellate swarmers (Fig. 2 D) are released by several of the zoidangia (Fig. 2 C). About 4-5 hours later other zoidangia liberate quadriflagellate swarmers (Fig. 2 E). No copulation phenomena could be observed. Although there were no seasonal changes in culture conditions, the relation of biflagellate to quadriflagellate swarmers changed in the course of the year favouring the latter in summer. Both types of swarmers are pear-shaped and have a lateral stigma (Fig. 2 F), but differ considerably in their sizes. The biflagellate swarmers measure up to 14.8 µm in length and 7.6 µm in breadth, the quadriflagellate swarmers are reaching 19.9 and 11.8 µm, respectively. Within 1 hour after liberation the swarmers throw off their flagella, settle down on the bottom of petri dishes and begin to germinate 1-3 days later. Germlings of biflagellate swarmers present good vegetative growth and morphological agreement with the mother thalli. In contrast, germlings of quadriflagellate swarmers show only little growth (even under different culture conditions). Up to this moment [redacted] could not raise adult thalli.

Nuclear DNA contents of starting material, biflagellate swarmers and their germlings are given in Fig. 3 (A, B, D). DNA content ranges of all these life cycle phases indicate identical ploidy levels. In the starting material there is one peak between 23.3 and 29.3 AU interpreted as G_1 stage of the mitotic cycle. Accordingly, the G_2 stage achieves DNA levels between 53.1 and 59.1 AU (Fig. 3 A).

Source : MNHN, Paris

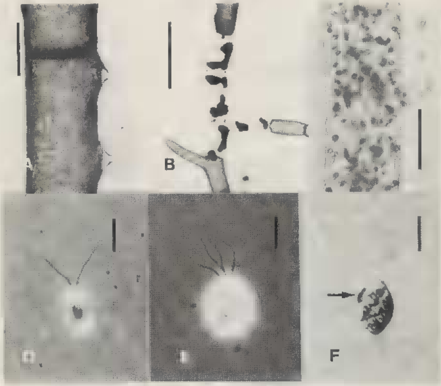

Fig. 2 — *Boodlea composita*, A. Development of liberation tubes in cells of reticulate frond before maturation; B. Zoidangia showing a network arrangement of protoplasma, from which zoospores are being formed; C. Liberation of zoospores through an opened ostiole; D. Biflagellate zoospore (glutaraldehyd fixation = GA); E. Quadriflagellate zoospore (GA); F. Quadriflagellate zoospore (GA) with lateral stigma. Phase contrast (C, D, E) or interference contrast (F); bar represents 100 μm (A, C), 500 μm (B), or 10 μm (D, E, F).

The nuclear DNA content of biflagellate swarmers (29.3-35.2 AU; Fig. 3 B) corresponds to the G_1 stage in the starting material. So, these swarmers are formed after a mitosis.

Nuclear DNA contents of quadriflagellate swarmers (Fig. 3 C) were found to be about half as high as measured in G_1 stage nuclei of the starting material (Fig. 3 A). The quadriflagellate swarmers are considered to have haploid nuclei in the G_1 stage. In consequence, the nuclei of cloned starting material and biflagellate swarmers released by these thalli are diploid.

Although it was still not possible to observe the complete life cycle of *Boodlea composita*, we believe to have good evidence for the existence of an alterna-

Source : MNHN, Paris

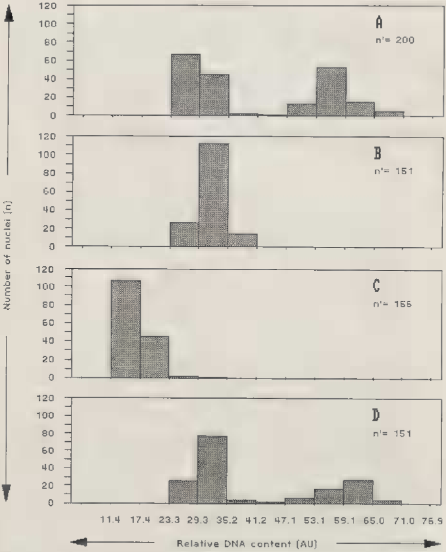

Fig. 3 — *Boodlea composita*, nuclear DNA contents (see Fig. 1). A. Diploid thalli with nuclei mainly in the G_1 and G_2 stages of the mitotic cycle; B. Diploid biflagellate mitozoospores; C. Haploid quadriflagellate meiozoospores; D. Germlings of B.

tion of two generations in this species. The diploid sporophytic generation (starting material) produces biflagellate mitozoospores and quadriflagellate meiozoospores. The function of the diploid biflagellate zoospores is the direct repro-

Source : MNHN, Paris

duction of the sporophyte, while haploid quadriflagellate zoospores presumeably develop into haploid gametophytic thalli. The poor growth of germlings of quadriflagellate zoospores under culture conditions favourable for diploid life cycle phases indicates that at least a physiological difference between both generations is existing.

It seems that in Siphonocladales the formation of diploid biflagellate zoospores is not related to certain life history types because this type of zoospores has been found in a species with haplobiontic life cycle and gametic meiosis *(Ernodesmis)* as well as in a most probably diplobiontic cycle with alternate sporophytic and gametophytic generations *(Boodlea)*.

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Source : MNHN, Paris